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Primary ciliary dyskinesia with normal ultrastructure: 3D tomography detects absence of DNAH11

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Take home message: 3D electron tomography identifies a defect in the cilia of patients with PCD and ‘normal ultrastructure’

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Abstract

In primary ciliary dyskinesia (PCD), motile ciliary dysfunction arises from ciliary defects usually confirmed by transmission electron microscopy (TEM). In 30% of patients, such as those with *DNAH11* mutations, apparently normal ultrastructure makes diagnosis difficult. Genetic analysis supports diagnosis but may not identify definitive causal variants. Electron tomography, an extension of TEM, produces 3D ultrastructural ciliary models with superior resolution to TEM. Our hypothesis is that tomography using existing patient samples will enable visualisation of *DNAH11*-associated ultrastructural defects. Dual axis tomograms from araldite-embedded nasal cilia were collected in 13 PCD patients with normal ultrastructure (7 *DNAH11*, 2 *HYDIN*, 3 *CCDC65* and 1 *DRC1*) and 6 healthy controls, then analysed using IMOD and Chimera software.

DNAH11 protein is localised to the proximal ciliary region. Within this region, electron tomography indicated a deficiency of >25% of proximal outer dynein arm volume in all patients with *DNAH11* mutations (n=7) compared to other patients with PCD and normal ultrastructure (n=6) and healthy controls (n=6). *DNAH11* mutations cause a shared abnormality in ciliary ultrastructure previously undetectable by TEM. Advantageously, electron tomography can be used on existing diagnostic samples and establishes a structural abnormality where ultrastructural studies were previously normal.

Introduction

Primary ciliary dyskinesia (PCD) is a genetic condition affecting approximately 1 in 15,000 of the population. Ineffective mucociliary clearance, caused by disruption of ciliary motility, leads to the clinical phenotype of chronic sino-pulmonary disease. (1) Symptoms frequently start in the neonatal period with neonatal respiratory distress and include chronic nasal discharge and wet cough, progressing in childhood to recurrent upper and lower airway infections and eventual bronchiectasis. (1) Cilia are also present in the embryonic node and defects in nodal cilia cause abnormalities of left-right laterality determination (situs) in ~50% patients with PCD. (1)

PCD is diagnosed using a combination of tests. These may include: the measurement of nasal nitric oxide, analysis of cilia function by high speed video microscopy and immunofluorescence analysis of protein expression (2, 3). PCD diagnosis is ultimately confirmed by identification of a hallmark defect of ciliary ultrastructure visualised by transmission electron microscopy (TEM) or detection of non-ambiguous bi-allelic mutations in a known PCD gene (2). Normal ciliary ultrastructure is shown in Figure 1. Patients with PCD most commonly have structural defects affecting the dynein arms, and/or microtubular arrangement (2, 4, 5). Apparently normal ultrastructure defined by conventional TEM is a common phenomenon and is thought to occur in up to 30% of cases (5-7). Genotyping also leaves 25-50% cases unsolved (2). In these cases, making a definitive diagnosis can be difficult.

Biallelic mutations in the *DNAH11* gene are a common cause of PCD accounting for at least 22% cases of PCD with 'normal TEM' ultrastructure results (8, 9). The

DNAH11 gene is thought to encode a heavy chain dynein protein component of the axonemal outer dynein arm (ODA) present in the proximal part of the cilium (Figure 1) (10). Surprisingly, despite its sizeable molecular mass (~520 kilodaltons), the consequences of high impact pathogenic mutations in *DNAH11* predicted to be loss-of-function causing absence of the protein (e.g. nonsense, frameshift and essential splice site mutations) are undetectable by TEM (9). This has long presented a diagnostic mystery, impeding definitive diagnosis, especially in cases where the identified *DNAH11* mutations are not previously reported or known to be disease-producing. PCD is genetically heterogeneous and although some common causal variants are found, the majority of patients - including those carrying *DNAH11* mutations - have unique mutations, some also classed as variants of unknown significance by international clinical variant calling and reporting standards (11). e.g. amino acid substitution 'missense' mutations of uncertain predicted pathogenicity (11). *DNAH11* mutations often confer a hyper-frequent ciliary beat visualised using high speed video microscopy. However this is not fully distinctive or diagnostic. Therefore, for patients with a *DNAH11* mutation, due to normal ultrastructure and the presence of significant retained ciliary motility by light microscopy, a diagnosis of PCD can be missed and, furthermore, when a diagnosis is suspected it can be difficult to confirm using the existing structural or functional tests.

Dual axis electron tomography is an extension of the traditional TEM technique. It allows visualisation of ciliary structures in three dimensions enabling enhanced resolution and understanding of the ciliary ultrastructure (12, 13). We previously used electron tomography to demonstrate absence of the C2b central pair projection as the downstream structural defect of *HYDIN* mutations (14). Electron tomography without

averaging has recently been shown in one patient to have potential to detect disturbance of cilia ultrastructure arising from *DNAH11* mutations (10).

In this study we test the hypothesis that despite normal ultrastructure using standard TEM, 3D reconstruction and sub-tomographic averaging using existing patient samples might enable visualisation of *DNAH11*- associated ultrastructural defects. We investigated whether there is a loss of volume in 3D of the ciliary ODA in *DNAH11* PCD compared to healthy controls and PCD with normal ultrastructure not caused by *DNAH11* mutations. In doing so, we aimed to investigate the potential of the 3D tomography technique for diagnosis and for advancing current knowledge of the effects on DNAH11 protein location of different *DNAH11* mutations.

Methods

Patient Selection

Seven patients, aged 7-18 years, with known biallelic disease causing mutations in *DNAH11* were selected. Details of subjects and a summary of their diagnostic investigations are shown in Table 1. The study was approved by the London Bloomsbury Research Ethics Committee under approval number 08/H0713/82.

ID	Sex	Age at TEM (yrs)	Clinical signs and symptoms	nNO (nl/min) (normal >77nl/min)	Light microscopy HSVM CBF (Hz)	TEM % dynein arm defects % MTD defects	<i>DNAH11</i> mutations	Genotype and predicted protein affect
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#1	M	18	Recurrent chest infections / rhinitis	29	Static 0 Hz	Normal DA 0% MTD 4%	[nonsense]; [frameshift]	[c.3727G>T]; [c.13531_13532insTTCAGGCTGAAGA] [p.Glu1243*]; [p.Ala4511Valfs*13]
#2	F	15	Recurrent chest infections / rhinitis	6	Static and hyperfrequent 22.6 Hz	Normal DA 0% MTD 5%	[missense]; [frameshift]	[c.7663C>T]; [c.4410_4413del] [p.Gln2555*]; [p.Ser1470Argfs*6]
#3	F	14	Situs inversus + recurrent chest infections	14	Static and hyperfrequent 12.8 Hz	Normal DA 0% MTD 7%	[nonsense]; [nonsense]	[c.5506C>T]; [c.5636T>A] [p.Arg1836*]; [p.Leu1879*]
#4	F	7	Recurrent chest infections / rhinitis	14	Slow with reduced bending 6.15 Hz	Normal DA 3% MTD 9%	[frameshift]; [frameshift]	[c.2832dup]; [c.13240dup] [p.Gln945Serfs*10]; [p.Thr4414Asnfs*34]
#5	F	10	Recurrent chest infections / rhinitis	50	Static and hyperfrequent (ND)	Normal DA 0% MTD 3%	[missense]; [missense]	[c.6506C>T]; [c.6506C>T] [p.Ser2169Leu]; [p.Ser2169Leu]
#6	F	14	Situs inversus + recurrent chest infections	20	Static and hyperfrequent 13.3 Hz	Normal DA 0% MTD 4%	[nonsense]; [nonsense]	[c.3220G>T]; [c.13090C>T] [p.Glu1074*]; [p.Arg4364*]
#7	M	9	Recurrent chest infections / rhinitis	8	Hyperfrequent 19.6 Hz	Normal DA 0% MTD 19%	[nonsense]; [splice site]	[c.3544C>T]; [c.8798-5G>A] [p.Arg1182*]

Table 1: Clinical details and diagnostic test results, including genotype, of the subjects with primary ciliary dyskinesia due to mutations in *DNAH11* (Gene encoding for Dynein Heavy Chain 11). M=male, F=female. TEM = transmission electron microscopy. HSVM = high speed video microscopy. nNO = nasal Nitric Oxide. DA = dynein arm defects, MTD = microtubular defects. ND= not done, due to dyskinesia frequency measurements were not taken. c.13531_13532insTTCAGGCTGAAGA mutation has not been proven for pathogenicity.

Two comparator groups were used. Group one consisted of six healthy controls without PCD. Group two consisted of six subjects with a diagnosis of PCD and normal TEM, not carrying *DNAH11* mutations. These cases were due to bi-allelic mutations in *HYDIN* (n=2), *CCDC65* (n=3) and *DRC1* (n=1). The PCD comparator group was chosen because ultrastructure is often considered normal in these cases, but it is not anticipated nor has it ever been seen that the ODA is affected by mutations in these three genes (14-17). These gene mutations are associated with isolated defects of the c2b central pair projection (*HYDIN*) and nexin-dynein regulatory complex (*CCDC65*, *DRC1*). A subject with a loss of function mutation in *DNAH5* with a known outer dynein arm defect by TEM was used as a negative control.

Electron tomography sample preparation

Ultrathin sections (~150nm) were cut using an ultra-microtome from blocks of nasal brushings embedded in araldite. Sections were stained with 2% uranyl acetate and lead citrate and subsequently labelled with 10nm gold fiducial markers.

The following areas (plane of sections are shown in Figure 1C) were assessed by tomography:

1. Longitudinal sections of the axoneme at the proximal portion of the cilium
2. Longitudinal sections of the axoneme at the distal portion of the cilium
3. Transverse cross sections, at least 6 per field of view, at the proximal portion of the axoneme (in the region of the microvilli).

Generation of electron tomograms

A JEOL 1400+ TEM was used to collect a series of images at different stage tilts. 4K by 4K digital images at 20,000X magnification were captured using JEOL

tomography software and a digital camera (AMT 16X, Deben Ltd, UK). The software automatically adjusts stage position, focus and exposure. The specimen was tilted through angles of $+65^{\circ}$ to -65° and images acquired at increments of 1° . Following the generation of images the sample was manually rotated 90° and in the same region a second series of images was acquired. The two image series were aligned and combined to produce a dual axis reconstruction using IMOD software (18, 19).

Sub-tomographic averaging

Sub-tomographic averaging was conducted to enhance the ODA structure using PEET (Particle Estimation for Electron Tomography), part of the IMOD software package (19). The regions chosen for averaging of longitudinal tomograms were at 96nm intervals (20). For transverse cross sections the centre of the A microtubule of each microtubule doublet (Figure 1) was selected as the centre for averaging. For quality control, averages in which the inner dynein arm and radial spokes could be visualised were considered acceptable for further analysis.

Tomography data analysis

Imaging, modelling and quantification of the ODAs was performed using the UCSF Chimera program developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California (supported by NIGMS P41-GM103311). The volume of averaged microtubule doublets and ODA was determined by a single individual blinded to details of the case using a bounding box as shown in Figure 4C. From these measurements, the percentage of the microtubule doublet that the ODA occupied was calculated (supplementary methods contain further information).

Immunofluorescence

Immunofluorescence labelling was performed as previously described (3, 21).

Imaging was conducted with a confocal microscope (SP5 Leica). (Full method in the online supplement).

Results

***DNAH11* longitudinal tomograms demonstrate a loss of volume from the ODA in the proximal cilia region**

In light of evidence indicating different ODA types in the proximal and distal cilium (10) (Figure 1C) we created averaged longitudinal tomograms from the proximal and distal regions of the ciliary axoneme from subjects with *DNAH11* mutations.

Tomograms obtained from the proximal region all showed a loss of ODA volume. A representative image of a 96 nm segment of the proximal ciliary microtubule containing four ODAs is shown in Figure 2A, comparing subject #4 who carries two frameshift loss-of-function *DNAH11* alleles to a healthy control. Quantification of arm volume in 4 *DNAH11* subjects in comparison to controls without respiratory disease (n=3) shows that approximately 2/3rds of the ODA volume is lost in individuals with *DNAH11* defects (Figure 2B) ($p<0.01$). Tomograms from the remaining 3 *DNAH11* subjects and 3 controls were not collected due to the technical difficulty of longitudinal electron tomography.

The retained structures seen in the proximal ODA using tomography were investigated further. In the tomogram images, it appears that a heavy chain head is

retained in each ODA, presumably devoid of DNAH11 but retaining DNAH5 protein as well as the light chain proteins associated with DNAH11 such as NME8 (Figure 2B). This was confirmed by immunofluorescence antibody staining from two DNAH11-deficient subjects (*DNAH11* #2 and #7) in whom the ODA heavy chain dynein DNAH5 (ODA head component) and axonemal light chain dynein NME8 (a component predicted to be closely associated with the ODA β heavy chain stalk) were both intact (Figure 3B, D).

DNAH11 is localised to the proximal region of the cilium and tomograms of the distal region appear normal.

No difference was seen in longitudinal tomograms from the distal region of the cilia in 3 subjects with *DNAH11* mutations, compared to 2 control subjects (Figure 2A, 2B). By immunofluorescence we confirmed a proximal axonemal localisation for DNAH11 in cilia from healthy controls with no protein present in the distal cilium (Figure 3A, C).

In keeping with this finding the ciliary beat pattern in *DNAH11* subjects, as assessed by high speed video microscopy, demonstrated a higher incidence of dyskinesia in the proximal, compared to the distal, region of the cilia. In six of seven samples there was reduced bending at the base of the cilium and preserved waveform at the tip, (supplementary video 1 and Table 1) compared to healthy controls (supplementary video 2). The incomplete beat in *DNAH11* subjects results in an apparently hyperfrequent ciliary beat, as previously reported, presumably due to reduced bend and therefore a shorter effective and recovery stroke, resulting in more beats per

second (8, 9). We speculate that this results from a lack of DNAH11 in the proximal cilium whilst the DNAH9- containing top (distal) half of the cilium is still moving. Taken together, high speed video microscopy, tomography and immunofluorescence studies confirm that DNAH11 is localised to the proximal portion of the cilium and consequently all further tomograms were taken from this region.

Transverse tomograms consistently show a loss of ODA volume in *DNAH11* subjects compared to controls

Longitudinal tomograms were found to have a high rate of technical failure (14 of 26 tomograms) and consequently could not routinely be used. Transverse cross sectional (Plane shown in Figure 1C) tomograms were trialled to produce a more robust methodology. All the transverse tomograms collected (N= 19) contained dynein arms and could be successfully analysed. A reduction of volume of the ODA was identified in all 7 *DNAH11* subjects compared to healthy controls (Figure 4A). The ODA proportion of the microtubular doublet had a median (IQR) of 10.3% (9.3-10.5) in *DNAH11* subjects compared to 13.8% (12.9-14.4) in healthy controls. Subjects with PCD caused by other defects (*HYDIN*, *CCDCD65* and *DRC1* mutations) had a median (IQR) ODA proportion of the microtubular doublet of 14.4% (13.1 – 16.0), similar to healthy controls. There was no overlap in the level of ODA volume loss between the *DNAH11* group and either control group (Figure 4A) indicating a significant loss of ODA volume arising from *DNAH11* mutations ($p < 0.01$). A subject with a homozygous nonsense mutation in *DNAH5* (c.13285C>T; p.Arg4429*) was included as a negative control. The ODA proportion in the proximal region was 2.4%, a value below the range of the *DNAH11* and other control subjects.

3D image analysis showed that the volume reduction in *DNAH11* subjects was from the ODA ‘forearm’ section of the tomogram (Figure 4B, supplementary movies 3 and 4). This was found consistently across all the tested subjects carrying different mutational variants within the *DNAH11* gene (green annotation, Figure 4B).

Discussion

We show that *DNAH11* mutations result in a characteristic abnormality of the ciliary ultrastructure detectable by electron tomography but not traditional TEM. The defect is specific to the ‘forearm’ of the ODA and is detected only at the proximal portion of the cilium where *DNAH11* is located.

The transverse section electron tomography technique used in this study is advantageous in that it uses existing fixed embedded samples already stored for diagnosis by standard TEM, thereby avoiding additional sampling. A strength of the technique used in this study is the quantification of results and the use of the MTD volume as an internal control during quantification, to minimise bias introduced by image analysis, such as manual selection of image features and thresholding.

Quantification showed no overlap in ODA volume measurements between healthy or PCD controls and *DNAH11* subjects, confirming the clear-cut nature of the underlying defect at this imaging resolution. The reliability of the test results suggest the technique has potential use in the diagnostic pathway for highly atypical PCD cases.

Using immunofluorescence and electron tomography we demonstrate that *DNAH11* mutations do not result in loss of the entire ODA. We found that a partial heavy chain of the ODA head region (presumed to be the DNAH5 protein), along with at least part of the remaining ODA structure (NME8 positive), remains intact along the length of the proximal cilia region in patients with *DNAH11* mutations. The localisation of these retained structures, coupled with the proximal nature of the DNAH11 loss, provides the likely explanation for the longstanding puzzle of apparently normal cilia structure in DNAH11 patients when using conventional TEM. Figure 5 shows a cartoon to demonstrate the predicted model of the ODA loss in *DNAH11* cases. These data arising from three dimensional (3D) tomographic imaging therefore advances our understanding of the cell biology of PCD disease and how it affects the cilia, such that the majority of structures remain completely undisturbed whilst a highly specific loss of only a portion of the ODA is sufficient to cause ineffective mucociliary clearance and its clinical consequences.

In this study we have shown that transverse section electron tomography can differentiate between *DNAH11* and control cases without the need for more complex longitudinal section or cryo-electron tomography techniques. The proximal location of DNAH11 means appropriate selection of a proximal area for transverse electron tomography is crucial for this technique. In the present study the presence of microvilli was used as a marker for proximity to the cell surface and therefore the DNAH11 region, however this method is imperfect, as occasional tip structures from bent cilia could reside in this region. The clear distinction between control and *DNAH11* cases suggest this was not a major limitation in defining a *DNAH11* defect in the present study. Other limiting factors of the electron tomography technique is

that it is time-consuming, labour intensive and requires specialist training. It can take days to collect, reconstruct and average each tomogram, resulting in only a small number of cross section being assessed and precluding its use as a high throughput diagnostic screening tool. However, high throughput was never our intention, and this technique could be reserved for specialist cases at a small number of reference centres since specimens which have already been embedded can easily be transported between centres. Use of chemically embedded sections and the restricted tomographic tilt range (± 65 rather than ± 90) are both factors which limit the full potential of tomographic resolution. The majority of the DNAH11 protein volume when imaging the ODAs in transverse section is in the depth (z-direction), which results in loss of resolution compared to image acquisition in the longitudinal plane. Use of longitudinal sections or cryo-electron tomography would overcome some of the resolution limitations and could thereby further elucidate the structural role of human DNAH11 (20). However, we found longitudinal tomography technically challenging and were not able to collect a full dataset using this technique (Figure 2B). Cryo-electron tomography is not practical for diagnostic evaluation in clinic or for the review of existing, previously unsolved, clinical samples, due to the requirement for a cryo electron microscope and the need for immediate preparation of samples following biopsy or culture (12).

Immunofluorescence can be used for confirmation of a DNAH11 defect. However, antibodies for DNAH11 are not widely available and others have shown that the protein remains present in cilia from individuals with PCD caused by pathogenic *DNAH11* missense mutations since the staining is indistinguishable from controls (10). One individual (Subject #5) in the present study carries a homozygous missense

mutation. Cilia from this subject demonstrate the same 25% tomographic volume loss as seen in the other 6 subjects carrying predicted null mutations (premature truncation and frameshift mutations). We speculate that the ability to detect protein loss arising from missense mutations by electron tomography is due to the superior resolution of the technique compared to immunofluorescence, however, this would need to be verified in further missense variants. It is possible that the difference in ODA volume could represent a conformational change in the dynein rather than a direct loss of the DNAH11 protein. This would provide another possible explanation for loss of volume in a missense mutation, where some protein is predicted to remain present.

Interestingly, Dougherty et al and data in this study have shown that DNAH9 can enter the proximal area of the axoneme in the absence of DNAH11 [14]. Our study suggests that any such replacement protein, still does not fully occupy the structural defect left by DNAH11 protein.

This study therefore provides proof of concept that electron tomography can be used to confirm a diagnosis of PCD, where traditional TEM appears normal. We suggest electron tomography, due to the requirement for microscopy equipment and its time consuming nature, could be used in specialist centres in cases where other tests for PCD have been inconclusive and the genetic testing if available rests upon variants of uncertain significance that require functional confirmation (e.g. unpublished missense mutations). Supplementary information file 1 demonstrates an example case study where tomography was used to confirm a diagnosis in a patient with a missense variant in *DNAH11*.

In summary, we have shown a consistent ultrastructural defect in subjects that carry deleterious *DNAH11* mutations once thought to have ‘normal ultrastructure’. We have shown that the tomography technique has good resolution and accuracy for the detection of PCD defects in *DNAH11*-deficient patients with apparently normal ultrastructure. Cilia electron microscopy has facilitated the major advances in PCD. Improved resolution and 3D visualisation of tomography has promise to provide important cell biological insights into the causes and variability of PCD along with an improved understanding of the significance of novel gene mutations.

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Figure legends

Figure 1

(A) Schematic of 9+2 cilia axoneme ultrastructure in respiratory cells of a healthy individual, composed of nine peripheral microtubule doublets and a central microtubule pair. Each doublet contains an A and incomplete B microtubule, with associated proteins attached to the A microtubule that include radial spokes and inner and outer (boxed) dynein arms; nexin-dynein regulatory complexes link the peripheral doublets. The dynein arms contain heavy chain dyneins, ATPases that hydrolyse ATP to provide energy for microtubule sliding and ciliary motility. One or more heavy chain forms the 'head' of the dynein arm which is attached to the dynein docking complex, which in turn is attached to the doublet A microtubule. (B) The outer dynein arms contain two heavy chains primarily responsible for its motor action to drive ciliary movement, which are thought to be attached to the microtubules by a docking complex. Three different outer dynein axonemal heavy chains are recognised in human respiratory cilia, DNAH5, DNAH9 and DNAH11. (C) In human nasal epithelial cell cilia, immunofluorescence studies show that DNAH5 localises along the whole axoneme whereas DNAH9 localises only to the distal axoneme whilst DNAH11 occupies only the proximal axoneme (closer to the cell membrane and microvilli). Thus DNAH9 and DNAH11 act as the second heavy chain to DNAH5 in different regions. Dotted lines indicate the longitudinal and transverse section planes.

Figure 2

(A) Representative examples of averaged, rendered and false coloured longitudinal electron tomograms of a single 96 nm repeating section of the cilia axoneme indicating

the outer dynein arms (blue) and A-tubule (grey): each 96 nm repeat contains four outer dynein arms. Tomograms show 4 outer dynein arms from the proximal axoneme of a healthy control (top image), and of patient #4 who carries two frameshift *DNAH11* mutations, taken from the distal region of the axoneme (middle image) and proximal region (bottom image). Red arrows indicate locations of the volume loss in the proximal axoneme of the *DNAH11* patient. White dashed lines indicate the 24nm repeat of each outer dynein arm. Retained structures are likely to represent DNAH5 and other light and intermediate chains of the outer dynein arm including NME8, a protein thought to be closely associated with the DNAH11 heavy chain. A quantification of the relative volume of the remaining structures is shown in Figure 2B. (B) Percentage contribution of the outer dynein arm to the microtubular doublet volume calculated from individual averaged tomograms from nasal brushings of *DNAH11* mutation PCD patients #1, #4, #6 and #7 compared to healthy controls (NC). Data from patients #2, #3 and #5 and the remaining controls could not be obtained despite multiple attempts due to technical difficulties with the longitudinal tomography and averaging technique in plastic section. The chart shows a reduction in outer dynein arm volume in *DNAH11* patients in the proximal region of the axoneme, but similar volumes between patients and controls in the distal region of the cilium.

Figure 3

(A) Main image; Immunofluorescence showing a proximal axoneme location for DNAH11 in a healthy nasal epithelial cell (green), with DNAH5 running the whole length of the cilium (red). Inset images show the top of two further ciliated cells, again showing the proximal location of DNAH11 (green) with acetylated tubulin (red, top image) running the length of the cilia and DNAH9 (red, bottom image) in the distal

region. White arrows indicate the position of DNAH11 throughout. B) High-resolution immunofluorescence from patient#2 who carries a frameshift and premature truncation mutation in the gene. DNAH11 (green) is absent from the cilia. Whereas DNAH5 (main image), acetylated alpha tubulin (top left insert) and DNAH9 (bottom left insert) are present throughout the length of the cilia (shown in red). (C) An alternative antibody to DNAH11 (red) was used to validate results (Abcam ab126571) and to show presence of DNAH11 in the peripheral airways. Cilia are marked in green with acetylated tubulin. Ciliated epithelium was obtained from a lung resection. D) NME8, a protein predicted to be closely associated with the β dynein heavy chain is shown to be retained (red D, right image) in an individual with PCD. Cilia are marked by acetylated alpha tubulin antibody (green). (D, left image)

Figure 4

(A) Box and whisker chart shows data from volume measurements from averaged transverse tomograms from the proximal portion of the axoneme from 6 healthy controls, 7 *DNAH11* mutation patients and 6 subjects with PCD due to other defects (*HYDIN* n=2, *CCDC65* n=3, *DRC1* n=1). There is a significant difference between the *DNAH11* mutation group and the two control groups. *p<0.01 (Mann-Whitney U test). (B) Representative transverse section tomograms averaged, rendered and false coloured, showing two different angles for each case. The top panel shows the tomographic average of a microtubular doublet, coloured blue, from a healthy control. Predicted locations of DNAH11 and DNAH5 are marked. The middle panel shows tomographic averages from *DNAH11* mutation patient#1 who carries a premature truncation mutation and frameshift mutation, the bottom panel shows tomographic averages from *DNAH11* mutation patient #2 who carries a different premature

truncation mutation and frameshift mutation. In the left boxes (middle and bottom), the predicted location of DNAH5 is coloured red. In the right boxes (middle and bottom), the green triangles have been added to the images to indicate where DNAH11 is predicted to be missing. (C) Tomograms from a healthy control as visualised in Chimera showing the bounding box method for quantification of the microtubular doublets (top) and the outer dynein arm (bottom). The percentage volume of the ODA out of the total MTD volume is then calculated and these values are shown in (A).

Figure 5

(A) Electron micrographs showing normal cilia ultrastructure in nasal brushings from a healthy control (left) and *DNAH11* mutation patient #1 (right). Below the micrographs are simplified models of the expected appearance of a DNAH11 defect in transverse section (B) and longitudinal section (C). Blue shapes represents the outer dynein arm and its docking complex, dotted white shapes the inner dynein arm and grey the A-tubule of the microtubular doublet

Footnotes

Table 1: Clinical details and diagnostic test results, including genotype, of the subjects with PCD due to mutations in *DNAH11*

Supplementary video 1: DNAH11 high speed video microscopy of cilia beating recorded at 500fps and played back at 30fps

Supplementary video 2: Healthy control high speed video microscopy of cilia beating recorded at 500fps and played back at 30fps

Supplementary video 3: Tomogram of DNAH11 transverse section

Supplementary video 4: Tomogram of control transverse section

Supplementary materials and methods

Supplementary results: Case study